

SEQUENCE LISTING

<110> The Curators of the University of Missouri

<120> LARGE SCALE EXPRESSION AND PURIFICATION OF RECOMBINANT
PROTEINS

<130> UMO1531.1

<140>

<141>

<150> 60/218,125

<151> 2000-01-13

<160> 2

<170> PatentIn Ver. 2.1

<210> 1

<211> 4087

<212> DNA

<213> Bos taurus

<220>

<221> CDS

<222> (268)..(3180)

<220>

<221> sig_peptide

<222> (268)..(363)

<220>

<221> misc_feature

<222> (3178)

<223> A Poly (H) affinity tag comprising 6 His residues
have been inserted at the C-Terminus end of the
coding region of the protein

<400> 1

gaattcggga gaagccatca ccccggtct tttttcacat ccagcccatg cagaccgatc 60

ggccagctca accagagctg ccaactgatct tccacactta agcaaaccac accagtgggt 120

ggcgaacatc aactcgtgct tgaaaaatac caacttggag cccggtttga gaagctacat 180

cagagtctcg agatgcgacg ctacaatctg cagttttcac tagcttccca gtaggttggg 240

10002010770000

```

acagttggaa ctctgccatt gcccagc atg ctg cag ttc agt ctg tca ccc acc 294
Met Leu Gln Ph Ser Leu Ser Pro Thr
1 5

ttg tcg atg gga ttt cac gtg ata gcc atg gtg gct ctc ttg ttt tcc 342
Leu Ser Met Gly Phe His Val Ile Ala Met Val Ala Leu Leu Phe Ser
10 15 20 25

cat gtg gac cat ata agt gct gag aca gaa atg gaa gga gaa ggc aac 390
His Val Asp His Ile Ser Ala Glu Thr Glu Met Glu Gly Glu Gly Asn
30 35 40

gag act ggc gag tgt act ggc tcc tat tac tgt aag aag ggg gtg att 438
Glu Thr Gly Glu Cys Thr Gly Ser Tyr Tyr Cys Lys Lys Gly Val Ile
45 50 55

tta ccc att tgg gag ccc cag gac cct tcc ttt gga gac aaa att gct 486
Leu Pro Ile Trp Glu Pro Gln Asp Pro Ser Phe Gly Asp Lys Ile Ala
60 65 70

aga gcg act gtg tat ttt gtg gcc atg gtc tac atg ttt ctt gga gtc 534
Arg Ala Thr Val Tyr Phe Val Ala Met Val Tyr Met Phe Leu Gly Val
75 80 85

tca atc att gct gac cgg ttc atg tcc tct ata gaa gtc atc acg tct 582
Ser Ile Ile Ala Asp Arg Phe Met Ser Ser Ile Glu Val Ile Thr Ser
90 95 100 105

caa gag aaa gaa atc acc ata aag aaa ccc aat gga gag acc acc aag 630
Gln Glu Lys Glu Ile Thr Ile Lys Lys Pro Asn Gly Glu Thr Thr Lys
110 115 120

aca act gtg agg atc tgg aat gag aca gtg tcc aac ctg acc ttg atg 678
Thr Thr Val Arg Ile Trp Asn Glu Thr Val Ser Asn Leu Thr Leu Met
125 130 135

gcc ctg ggg tct tca gct cca gag att ctc ctt tca gta atc gag gtg 726
Ala Leu Gly Ser Ser Ala Pro Glu Ile Leu Leu Ser Val Ile Glu Val
140 145 150

tgt ggc cat aac ttc act gca gga gac ctt ggc cct agc acc atc gtg 774
Cys Gly His Asn Phe Thr Ala Gly Asp Leu Gly Pro Ser Thr Ile Val
155 160 165

ggg agt gct gca ttc aac atg ttc atc atc att gcc ctt tgt gtg tat 822
Gly Ser Ala Ala Phe Asn Met Phe Ile Ile Ile Ala Leu Cys Val Tyr
170 175 180 185

```


agg	cat	gca	gca	gac	caa	gcc	agg	aaa	gct	gtc	agc	atg	cat	gag	gtc	1446
Arg	His	Ala	Ala	Asp	Gln	Ala	Arg	Lys	Ala	Val	Ser	Met	His	Glu	Val	
		380					385					390				
aac	acg	gaa	gtg	gct	gaa	aat	gac	cct	gtc	agt	aag	atc	ttc	ttt	gaa	1494
Asn	Thr	Glu	Val	Ala	Glu	Asn	Asp	Pro	Val	Ser	Lys	Ile	Phe	Phe	Glu	
		395				400					405					
caa	ggg	aca	tat	cag	tgt	ctg	gag	aac	tgt	ggc	aca	gta	gcc	ctg	acc	1542
Gln	Gly	Thr	Tyr	Gln	Cys	Leu	Glu	Asn	Cys	Gly	Thr	Val	Ala	Leu	Thr	
410					415					420					425	
att	atc	cgc	aga	ggc	ggc	gat	ttg	acc	aac	act	gtg	ttt	gtt	gac	ttc	1590
Ile	Ile	Arg	Arg	Gly	Gly	Asp	Leu	Thr	Asn	Thr	Val	Phe	Val	Asp	Phe	
				430					435					440		
aga	aca	gag	gat	ggc	aca	gcc	aat	gct	gga	tct	gat	tac	gaa	ttt	acc	1638
Arg	Thr	Glu	Asp	Gly	Thr	Ala	Asn	Ala	Gly	Ser	Asp	Tyr	Glu	Phe	Thr	
			445					450					455			
gaa	gga	act	gtg	gtc	ttt	aag	cct	ggc	gag	acc	cag	aag	gaa	atc	aga	1686
Glu	Gly	Thr	Val	Val	Phe	Lys	Pro	Gly	Glu	Thr	Gln	Lys	Glu	Ile	Arg	
		460					465					470				
gtt	ggc	atc	att	gat	gat	gac	atc	ttt	gag	gag	gat	gag	aat	ttc	ctt	1734
Val	Gly	Ile	Ile	Asp	Asp	Asp	Ile	Phe	Glu	Glu	Asp	Glu	Asn	Phe	Leu	
	475					480					485					
gtg	cat	ctc	agc	aac	gtc	aaa	gta	tct	ttg	gaa	gcc	tcg	gaa	gac	ggc	1782
Val	His	Leu	Ser	Asn	Val	Lys	Val	Ser	Leu	Glu	Ala	Ser	Glu	Asp	Gly	
490					495					500					505	
atc	ctg	gaa	gcc	agt	cat	gtc	tct	acc	ctt	gct	tgc	ctg	gga	tcc	ccc	1830
Ile	Leu	Glu	Ala	Ser	His	Val	Ser	Thr	Leu	Ala	Cys	Leu	Gly	Ser	Pro	
				510					515					520		
tcc	act	gcc	acc	gtg	act	att	ttt	gat	gat	gac	cat	gct	ggc	atc	ttt	1878
Ser	Thr	Ala	Thr	Val	Thr	Ile	Phe	Asp	Asp	Asp	His	Ala	Gly	Ile	Phe	
			525					530					535			
act	ttt	gag	gaa	ccg	gtg	act	cat	gtg	agt	gag	agc	att	ggc	atc	atg	1926
Thr	Phe	Glu	Glu	Pro	Val	Thr	His	Val	Ser	Glu	Ser	Ile	Gly	Ile	Met	
		540					545					550				
gag	gtg	aaa	gtt	ctg	aga	aca	tct	gga	gca	cgt	gga	aat	gtt	atc	gtt	1974
Glu	Val	Lys	Val	Leu	Arg	Thr	Ser	Gly	Ala	Arg	Gly	Asn	Val	Ile	Val	
		555					560				565					

ccc tat aag acc att gag ggg acc gcc aga ggt gga ggg gag gac ttt	2022
Pro Tyr Lys Thr Ile Glu Gly Thr Ala Arg Gly Gly Gly Glu Asp Phe	
570 575 580 585	
 gag gac aca tgc gga gag ctc gag ttc cag aat gac gaa att gtc aaa	2070
Glu Asp Thr Cys Gly Glu Leu Glu Phe Gln Asn Asp Glu Ile Val Lys	
590 595 600	
 aca ata tca gtc aag gta att gat gat gag gag tat gag aaa aac aag	2118
Thr Ile Ser Val Lys Val Ile Asp Asp Glu Glu Tyr Glu Lys Asn Lys	
605 610 615	
 acc ttc ttc ctt gag att gga gag ccc cgc ctg gtg gag atg agt gag	2166
Thr Phe Phe Leu Glu Ile Gly Glu Pro Arg Leu Val Glu Met Ser Glu	
620 625 630	
 aag aaa gcc ctg tta ttg aat gag ctt ggt ggc ttc aca ata aca ggg	2214
Lys Lys Ala Leu Leu Leu Asn Glu Leu Gly Gly Phe Thr Ile Thr Gly	
635 640 645	
 aaa tac ctg tat ggc cag cct gtc ttc agg aaa gtt cat gct aga gaa	2262
Lys Tyr Leu Tyr Gly Gln Pro Val Phe Arg Lys Val His Ala Arg Glu	
650 655 660 665	
 cat cca ctc ccc tct act ata atc acc atc gca gat gaa tat gat gac	2310
His Pro Leu Pro Ser Thr Ile Ile Thr Ile Ala Asp Glu Tyr Asp Asp	
670 675 680	
 aag cag cca ctg acc agc aaa gag gag gaa gag agg cgc att gcg gaa	2358
Lys Gln Pro Leu Thr Ser Lys Glu Glu Glu Glu Arg Arg Ile Ala Glu	
685 690 695	
 atg ggg cgc ccc att ctg gga gag cac acc aga ctg gag gtg atc att	2406
Met Gly Arg Pro Ile Leu Gly Glu His Thr Arg Leu Glu Val Ile Ile	
700 705 710	
 gaa gaa tcc tac gag ttc aag agt acc gtg gac aaa ctg att aag aag	2454
Glu Glu Ser Tyr Glu Phe Lys Ser Thr Val Asp Lys Leu Ile Lys Lys	
715 720 725	
 aca aac cta gcc ctc gtg gtt ggg acg aac agc tgg aga gag cag ttc	2502
Thr Asn Leu Ala Leu Val Val Gly Thr Asn Ser Trp Arg Glu Gln Phe	
730 735 740 745	
 atc gag gcg atc act gtc agt gct ggg gaa gat gac gat gac gac gaa	2550
Ile Glu Ala Ile Thr Val Ser Ala Gly Glu Asp Asp Asp Asp Asp Glu	
750 755 760	

tgt ggg gag gag aag ctg ccc tcc tgt ttt gac tac gtg atg cac ttt	2598
Cys Gly Glu Glu Lys Leu Pro Ser Cys Phe Asp Tyr Val Met His Ph	
765 770 775	
ctg act gtg ttc tgg aag gtc ctc ttc gcc ttt gtc ccc ccg aca gag	2646
Leu Thr Val Phe Trp Lys Val Leu Phe Ala Phe Val Pro Pro Thr Glu	
780 785 790	
tac tgg aac ggc tgg gcg tgt ttc atc gtc tcc atc ctc atg atc ggc	2694
Tyr Trp Asn Gly Trp Ala Cys Phe Ile Val Ser Ile Leu Met Ile Gly	
795 800 805	
cta ctg acg gct ttc att gga gac ctc gct tcc cac ttc gcc tgc acc	2742
Leu Leu Thr Ala Phe Ile Gly Asp Leu Ala Ser His Phe Ala Cys Thr	
810 815 820 825	
atc gcc ctg aag gat tcc gtg acc gcg gtg gtg ttc gtt gcg ctt gga	2790
Ile Ala Leu Lys Asp Ser Val Thr Ala Val Val Phe Val Ala Leu Gly	
830 835 840	
acc tca gtg cca gac aca ttt gca agc aaa gtg gcc gcc acc cag gac	2838
Thr Ser Val Pro Asp Thr Phe Ala Ser Lys Val Ala Ala Thr Gln Asp	
845 850 855	
cag tat gcg gat gca tcc ata ggt aac gtc aca ggc agc aac gcg gtg	2886
Gln Tyr Ala Asp Ala Ser Ile Gly Asn Val Thr Gly Ser Asn Ala Val	
860 865 870	
aac gtc ttc ctg ggc atc ggt gtg gcc tgg tcc atc gcc gcc atc tac	2934
Asn Val Phe Leu Gly Ile Gly Val Ala Trp Ser Ile Ala Ala Ile Tyr	
875 880 885	
cac gcg gcc aac ggg gaa cag ttc aaa gtg tcc cct ggc acg cta gct	2982
His Ala Ala Asn Gly Glu Gln Phe Lys Val Ser Pro Gly Thr Leu Ala	
890 895 900 905	
ttt tct gtc act ctc ttc acc att ttt gct ttc atc aat gtg ggg gtg	3030
Phe Ser Val Thr Leu Phe Thr Ile Phe Ala Phe Ile Asn Val Gly Val	
910 915 920	
ctg ctg tat cgg cgg agg cca gaa att gga ggt gag ctg ggt ggg ccc	3078
Leu Leu Tyr Arg Arg Arg Pro Glu Ile Gly Gly Glu Leu Gly Gly Pro	
925 930 935	
cgg act gcc aag ctc ctc aca tcc tgc ctc ttt gtg ctc ctg tgg ctc	3126
Arg Thr Ala Lys Leu Leu Thr Ser Cys Leu Phe Val Leu Leu Trp L u	
940 945 950	

ttg tac att ttc ttc tcc tcc ctg gag gcc tac tgc cac ata aaa ggc 3174
 Leu Tyr Ile Phe Phe Ser Ser Leu Glu Ala Tyr Cys His Ile Lys Gly
 955 960 965

ttc taa aggaacaatc agatgtagta aatttatata tatatacata tatatatata 3230
 Phe
 970

cataaaaatt atgtataatg aacagaggaa actggcattt gtcattgtcca cccacctgct 3290

gatggaatcc agcttcaaga gcagactctg tactagggcc ggagagagaa ggcattcacct 3350

cccgtttccc aggggcgttc gtcttgttga accaggcatg gaggcagggc catctttacg 3410

tcagctcagc ccagaagcgg tgtgttctcc cggggttcat aaatccttaa gttctttgat 3470

ttgttttctg tttttgcttg ttttgggtcg gggtagggag gtgggttgatg ttagggtttg 3530

gttttggttt tgcaggggga agatcagggt ttgtggctct cttgtgggag gtgatgtcca 3590

atctcaatgg taaaaatgga aatcaggaag atgactctcc ctttgcccaa aaactttaaa 3650

aattattttg gagtaagaaa ggaaacgggc atggaagaag aaagaagcat gtcttcacca 3710

tattactaaa tttcatgcct tatctctgga gtgggagcag aggtgaagtc ctccctccaa 3770

gaagaaacag gggagctgga atggagccaa gaagagtcatt ggttctagat acagtctgat 3830

gtttaaagat acatcgctgc ctggcaccct tgttcaacag gtacaaaaac aacatgccta 3890

gattcccagg aacgcacaaa gtcctttctt atctcttcag cgctggactg tgattagcaa 3950

ggccctgatt ctgatgttct acaccgctg attccccagc cctcccatcc caaaccctt 4010

ctccggaccc tttacccttc gtacaaacag gaagaataac tccattcaaa aagcacacca 4070

tcctttccat tcgcattc 4087

<210> 2

<211> 970

<212> PRT

<213> Bos taurus

<400> 2

Met Leu Gln Phe Ser Leu Ser Pro Thr Leu Ser Met Gly Phe His Val

1

5

10

15

Ile Ala Met Val Ala Leu Leu Phe Ser His Val Asp His Ile Ser Ala

20																25						30					
Glu	Thr	Glu	Met	Glu	Gly	Glu	Gly	Asn	Glu	Thr	Gly	Glu	Cys	Thr	Gly												
35				40				45																			
Ser	Tyr	Tyr	Cys	Lys	Lys	Gly	Val	Ile	Leu	Pro	Ile	Trp	Glu	Pro	Gln												
50				55				60																			
Asp	Pro	Ser	Phe	Gly	Asp	Lys	Ile	Ala	Arg	Ala	Thr	Val	Tyr	Phe	Val												
65				70				75				80															
Ala	Met	Val	Tyr	Met	Phe	Leu	Gly	Val	Ser	Ile	Ile	Ala	Asp	Arg	Phe												
				85				90				95															
Met	Ser	Ser	Ile	Glu	Val	Ile	Thr	Ser	Gln	Glu	Lys	Glu	Ile	Thr	Ile												
				100				105				110															
Lys	Lys	Pro	Asn	Gly	Glu	Thr	Thr	Lys	Thr	Thr	Val	Arg	Ile	Trp	Asn												
115				120				125																			
Glu	Thr	Val	Ser	Asn	Leu	Thr	Leu	Met	Ala	Leu	Gly	Ser	Ser	Ala	Pro												
130				135				140																			
Glu	Ile	Leu	Leu	Ser	Val	Ile	Glu	Val	Cys	Gly	His	Asn	Phe	Thr	Ala												
145				150				155				160															
Gly	Asp	Leu	Gly	Pro	Ser	Thr	Ile	Val	Gly	Ser	Ala	Ala	Phe	Asn	Met												
				165				170				175															
Phe	Ile	Ile	Ile	Ala	Leu	Cys	Val	Tyr	Val	Val	Pro	Asp	Gly	Glu	Thr												
				180				185				190															
Arg	Lys	Ile	Lys	His	Leu	Arg	Val	Phe	Phe	Val	Thr	Ala	Ala	Trp	Ser												
195				200				205																			
Ile	Phe	Ala	Tyr	Thr	Trp	Leu	Tyr	Ile	Ile	Leu	Ser	Val	Ser	Ser	Pro												
210				215				220																			
Gly	Val	Val	Glu	Val	Trp	Glu	Gly	Leu	Leu	Thr	Phe	Phe	Phe	Phe	Pro												
225				230				235				240															
Ile	Cys	Val	Val	Phe	Ala	Trp	Val	Ala	Asp	Arg	Arg	Leu	Leu	Phe	Tyr												
				245				250				255															
Lys	Tyr	Val	Tyr	Lys	Arg	Tyr	Arg	Ala	Gly	Lys	Gln	Arg	Gly	Met	Ile												
260				265				270																			
Ile	Glu	His	Glu	Gly	Asp	Arg	Pro	Ser	Ser	Lys	Thr	Glu	Ile	Glu	Met												
275				280				285																			
Asp	Gly	Lys	Val	Val	Asn	Ser	His	Val	Asp	Ser	Phe	Leu	Asp	Gly	Ala												
290				295				300																			
Leu	Val	Leu	Glu	Val	Asp	Glu	Arg	Asp	Gln	Asp	Asp	Glu	Glu	Ala	Arg												
305				310				315				320															
Arg	Glu	Met	Ala	Arg	Ile	Leu	Lys	Glu	Leu	Lys	Gln	Lys	His	Pro	Glu												
				325				330				335															
Lys	Glu	Ile	Glu	Gln	Leu	Ile	Glu	Leu	Ala	Asn	Tyr	Gln	Val	Leu	Ser												
340				345				350																			
Gln	Gln	Gln	Lys	Ser	Arg	Ala	Phe	Tyr	Arg	Ile	Gln	Ala	Thr	Arg	Leu												
355				360				365																			
Met	Thr	Gly	Ala	Gly	Asn	Ile	Leu	Lys	Arg	His	Ala	Ala	Asp	Gln	Ala												
370				375				380																			
Arg	Lys	Ala	Val	Ser	Met	His	Glu	Val	Asn	Thr	Glu	Val	Ala	Glu	Asn												
385				390				395				400															
Asp	Pro	Val	Ser	Lys	Ile	Phe	Phe	Glu	Gln	Gly	Thr	Tyr	Gln	Cys	Leu												

405										410				415			
Glu	Asn	Cys	Gly	Thr	Val	Ala	Leu	Thr	Ile	Ile	Arg	Arg	Gly	Gly	Asp		
420										425				430			
Leu	Thr	Asn	Thr	Val	Phe	Val	Asp	Phe	Arg	Thr	Glu	Asp	Gly	Thr	Ala		
435										440				445			
Asn	Ala	Gly	Ser	Asp	Tyr	Glu	Phe	Thr	Glu	Gly	Thr	Val	Val	Phe	Lys		
450										455				460			
Pro	Gly	Glu	Thr	Gln	Lys	Glu	Ile	Arg	Val	Gly	Ile	Ile	Asp	Asp	Asp		
465										470				475			
Ile	Phe	Glu	Glu	Asp	Glu	Asn	Phe	Leu	Val	His	Leu	Ser	Asn	Val	Lys		
485										490				495			
Val	Ser	Leu	Glu	Ala	Ser	Glu	Asp	Gly	Ile	Leu	Glu	Ala	Ser	His	Val		
500										505				510			
Ser	Thr	Leu	Ala	Cys	Leu	Gly	Ser	Pro	Ser	Thr	Ala	Thr	Val	Thr	Ile		
515										520				525			
Phe	Asp	Asp	Asp	His	Ala	Gly	Ile	Phe	Thr	Phe	Glu	Glu	Pro	Val	Thr		
530										535				540			
His	Val	Ser	Glu	Ser	Ile	Gly	Ile	Met	Glu	Val	Lys	Val	Leu	Arg	Thr		
545										550				555			
Ser	Gly	Ala	Arg	Gly	Asn	Val	Ile	Val	Pro	Tyr	Lys	Thr	Ile	Glu	Gly		
565										570				575			
Thr	Ala	Arg	Gly	Gly	Gly	Glu	Asp	Phe	Glu	Asp	Thr	Cys	Gly	Glu	Leu		
580										585				590			
Glu	Phe	Gln	Asn	Asp	Glu	Ile	Val	Lys	Thr	Ile	Ser	Val	Lys	Val	Ile		
595										600				605			
Asp	Asp	Glu	Glu	Tyr	Glu	Lys	Asn	Lys	Thr	Phe	Phe	Leu	Glu	Ile	Gly		
610										615				620			
Glu	Pro	Arg	Leu	Val	Glu	Met	Ser	Glu	Lys	Lys	Ala	Leu	Leu	Leu	Asn		
625										630				635			
Glu	Leu	Gly	Gly	Phe	Thr	Ile	Thr	Gly	Lys	Tyr	Leu	Tyr	Gly	Gln	Pro		
645										650				655			
Val	Phe	Arg	Lys	Val	His	Ala	Arg	Glu	His	Pro	Leu	Pro	Ser	Thr	Ile		
660										665				670			
Ile	Thr	Ile	Ala	Asp	Glu	Tyr	Asp	Asp	Lys	Gln	Pro	Leu	Thr	Ser	Lys		
675										680				685			
Glu	Glu	Glu	Glu	Arg	Arg	Ile	Ala	Glu	Met	Gly	Arg	Pro	Ile	Leu	Gly		
690										695				700			
Glu	His	Thr	Arg	Leu	Glu	Val	Ile	Ile	Glu	Glu	Ser	Tyr	Glu	Phe	Lys		
705										710				715			
Ser	Thr	Val	Asp	Lys	Leu	Ile	Lys	Lys	Thr	Asn	Leu	Ala	Leu	Val	Val		
725										730				735			
Gly	Thr	Asn	Ser	Trp	Arg	Glu	Gln	Phe	Ile	Glu	Ala	Ile	Thr	Val	Ser		
740										745				750			
Ala	Gly	Glu	Asp	Asp	Asp	Asp	Asp	Glu	Cys	Gly	Glu	Glu	Lys	Leu	Pro		
755										760				765			
Ser	Cys	Phe	Asp	Tyr	Val	Met	His	Phe	Leu	Thr	Val	Phe	Trp	Lys	Val		
770										775				780			
Leu	Phe	Ala	Phe	Val	Pro	Pro	Thr	Glu	Tyr	Trp	Asn	Gly	Trp	Ala	Cys		

